A STUDY OF THE EFFECT OF PROTEIN IN TUBULAR FLUID IN PROXIMAL TUBULAR REABSORPTION

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A STUDY OF THE EFFECT OF PROTEIN IN TUBULAR FLUID IN PROXIMAL TUBULAR REABSORPTION

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TABLE OF CONTENTS

The classical thesis of how the kidney handled plasma proteins concurred with the work of Cushny in 1926 (1) who held to the concept that "the excretion of injected protein seems to occur only with injury to the glomerular capsule." Furthermore, it was thought that protein was neither reabsorbed nor secreted in the tubules. Since that time a large body of evidence has accrued which refutes both statements, showing that the glomerular membrane is indeed permeable to plasma proteins and that these proteins are at least partially reabsorbed in the proximal tubules.

DeHaan (2) was one of the first to show that proteins passed through the normal glomerular membrane although Ludwig had alluded to this concept when he described the glomerulus as being a mechanical filter in 1844. The chemist Berzelius noted protein in the urine of apparently normal people when they ingested raw eggs. Under normal circumstances human urine contains small amounts of serum proteins (3, 4), and Dock (5), utilizing the technique of Beckford and Winton of kidney perfusion with ice cold serum, obtained a "urine" containing 15-22 milligrams of protein per 100 cc.

It has been shown that smaller proteins pass the glomerular membrane with relative ease (6) whereas the membrane is less permeable to larger proteins. Egg albumin, Bence-Jones proteins and hemoglobin with a molecular weight of 68,000 are able to penetrate the glomerular membrane but the membrane is more restrictive to serum albumin, edestin and casein.

Walker et al analyzed specimens of glomerular filtrate and proximal tubule fluid and found that of 41 specimens, 16 were positive for protein; 14 of these contained less than 200 milligrams per cent and nine less than 80 milligrams per cent. Of the 25 negative specimens, 8 had at least less than 30 milligrams per cent and 17 less than 80 milligrams per cent (7). Subsequently it has been pointed out using electrophoretic methods that the protein in normal rat urine is alpha and beta globulins with little or no albumin present (8).

It is readily apparent with the large glomerular flow and yet low concentration of protein in urine (4) with essentially no albumin, that protein is reabsorbed as the glomerular filtrate passes down the tubule (9). Clearance studies using hemoglobin in dogs have enabled the calculation of the maximum tubular reabsorption of hemoglobin (10) and the threshold for loss of hemoglobin through the kidney (11). Using Evans Blue (T 1824) which binds with plasma albumin, Dock (5) showed that following intravenous administration to normal rats, blue droplets appeared in cells lining the proximal convoluted tubules (12, 13). Histochemical methods have been able to demonstrate the resorption of protein (14, 15) and other workers (16) have shown the presence of diffusely reabsorbed protein in the tubule of the rat using fluorescent antibodies to bovine serum albumin.

Recognition of the low concentration of protein in tubular fluid compared to plasma levels, prompts the question as to what part this high protein concentration gradient plays in the mechanism of salt and water reabsorption in the proximal tubule. It was the design of this experimental work to determine what effect serum proteins in tubular fluid, in the range of plasma levels, had on proximal tubular reabsorption as compared to the reabsorption of isotonic saline. In the present study the droplet method of micropuncture was used as described by Gertz (17). This technique enables one to visualize and photograph the change in distance between two oil droplets in a tubule as the intervening fluid is being absorbed. From the data obtained, the volume of solution resorbed per unit area of the tubule per second can be calculated and rates of reabsorption of various fluids can be compared.

METHODS

Male white rats weighing 150 - 275 mgm, fed a standard laboratory diet and adequately hydrated were anesthetized with Inactin*. The left kidney was isolated through a left upper quadrant transverse incision and, after removing the perinephric fat and capsule to the pedicle, the kidney was immobilized in a plexiglass boat and covered with paraffin oil. A flexible ribbon heating element overlaid the cork base which supported the rat and provided a constant body temperature.

A double barreled pipette with a leveled tip was used for the micropuncture. One lumen was filled with castor oil containing Sudan Black, the other with the fluid being studied. The tip of pipette was inserted into a proximal tubule at the surface of the kidney and the oil-Sudan Black mixture injected into the tubule. This oil column, readily visualized because of the Sudan Black, was then broken by injecting the solution under study from the other barrel of the pipette. After sealing off the tip of the pipette with a minute amount of oil injection, sequential photographs were taken.

An American Optical binocular dissecting microscope with Greenough optics was used to visualize the kidney and a Leitz micromanipulator was utilized to bring the pipette to the proper

* Promonta, Hamburg, Germany

position and puncture the tubule. Two 30 watt low voltage incandescent lamps and a 25 watt zirconium arc lamp illuminated the kidney. To one of the oculars of the microscope was attached a Robot Star II camera connected to an intervalometer which allowed sequential photographs at the rate of one every three seconds.

The reabsorption of three different solutions was studied in the proximal tubule; a saline solution containing 150 mEq per liter of sodium chloride, a 10% solution of rat serum diluted with isotonic saline and a mixture of isotonic rat serum and saline in the proportion of 1:1 (50% serum).

Enlarged prints at 2.25 magnification were made of the 35 mm film negatives. From the prints (see Figure 1) one could measure the original distance between the oil droplets and the subsequent change per three second interval.

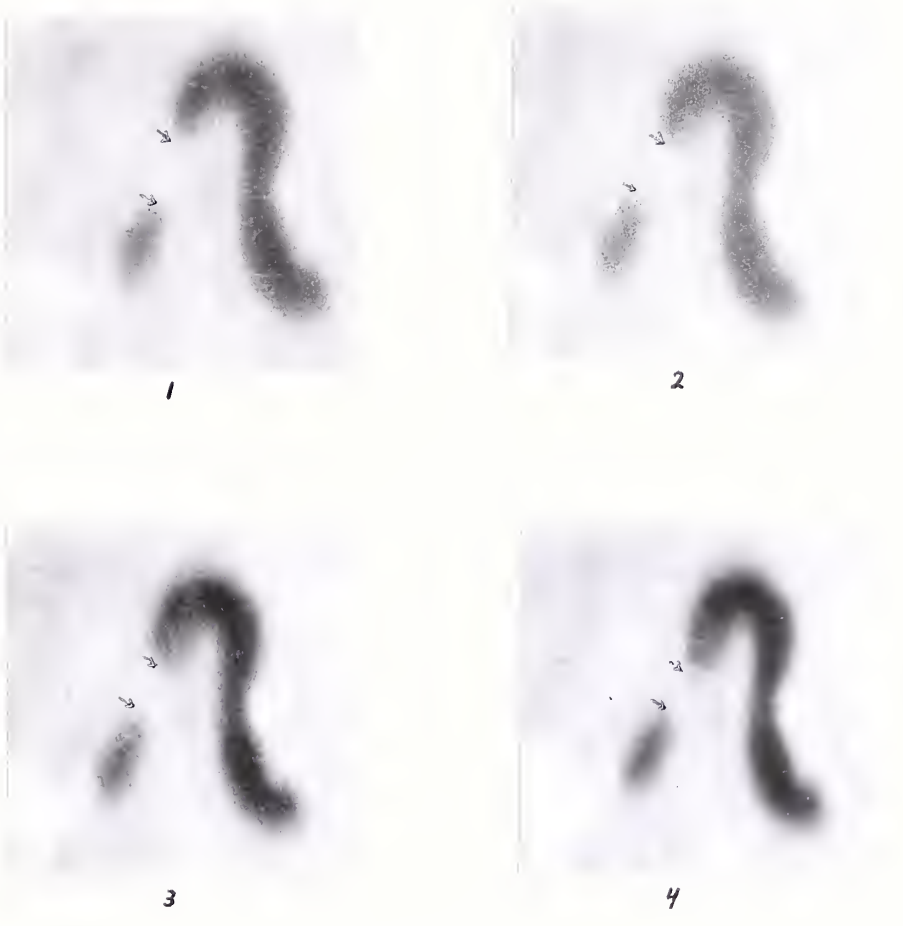

Figure 1: Sequential photographs of an injected tubule with resorption of fluid occurring between oil droplets. Arrows point to the change in distance between oil droplets with resorption.

RESULTS

The average half time for the resorption of a series of nine saline solutions (150 mEq/L) was 9.9 seconds with a standard deviation of .5 seconds. Subsequently, all the fluid was reabsorbed. Plotting the volume at time t over the volume at t = 0 (V/V_o) against time (Figure 2) on semi log paper, the curve approximated that of a straight line. This indicates that the saline solution was resorbed at a constant rate per unit area of tubular lumen. Using the formula $\emptyset Na = .347\ r/t_{\frac{1}{2}}$ (see discussion for derivation), where $\emptyset Na$ equals volume resorbed per unit area of tubular surface per second, $t_{\frac{1}{2}}$ equals the half-time for resorption and r equals the radius of the tubule, $\emptyset Na$ is found to equal $(5.4 \pm .3)\ 10^{-4}\ mm^3/mm^2/sec$ for isotonic saline.

In a series of six, the average half time resorption for a 10% rat serum (90% isotonic saline 10% rat serum) was 11.6 ± 1.1 (Table 1). Figure 3 illustrates a representative curve for the resorption of 10% serum. Using 50% serum, the average half time resorption in a series of nine was $15.1 \pm 1.5\ mm^3/mm^2/sec$ (Table 1). A representative curve of the reabsorption of 50% serum is shown in Figure 4. It is noted that the solutions were not completely resorbed during the time of observation when either the 10% or 50% serum solution was studied. With the 50% serum, equilibrium of

of efflux and influx was reached after approximately 70% of the volume at t = 0 was reabsorbed. It should be pointed out that more than 70% of the original volume has been resorbed when resorption appears to cease, because resorption has taken place during the lapse of time (approximately 2 - 3 seconds) from the injection of fluid and when the first picture was taken to determine V_o.

The value $\emptyset_{Na} = (5.4 \pm .3)\ 10^{-4}\ mm^3/mm^2/sec$ obtains at any time during the resorption of saline from T = 0 to complete resorption. However, $\emptyset$ is not constant when a protein solution is resorbed but proportional to the slope of the curve when V/V_o is plotted against time. (Figure 3 and 4) The formula $\emptyset = \frac{r}{2} \frac{\ln V_2/V_1}{t}$ (see discussion for derivation) allows us to calculate the average rate of resorption of a protein solution during a small interval of time. We have arbitrarily calculated $\emptyset$ over a 3 second interval where V_1 equals the volume at the beginning and V_2 the volume at the end of the three seconds. Table 2, and Table 3 give the results of such determinations and shows how the rate, $\emptyset$, of resorption decreases as the original amount of fluid injected decreases with resorption. The initial rate of resorption of both 10% and 50 serum is in close agreement with the rate of resorption of isotonic saline.

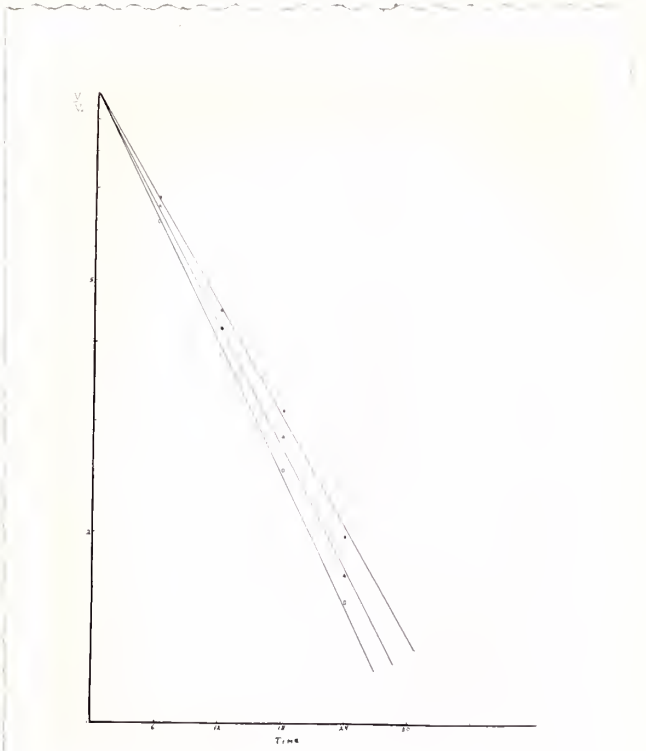

Figure 2: Resorption Curve for Isotonic Saline.

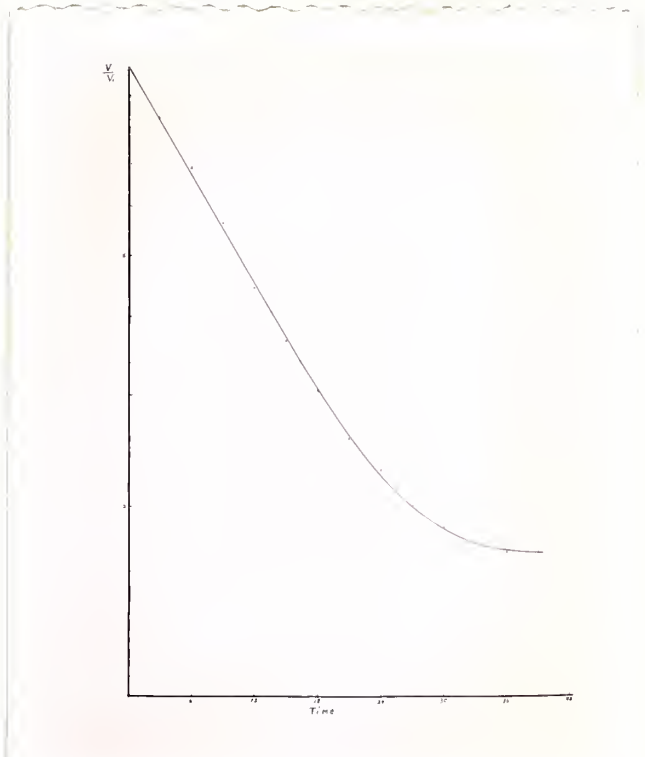

Figure 3: Resorption Curve for 10% Serum.

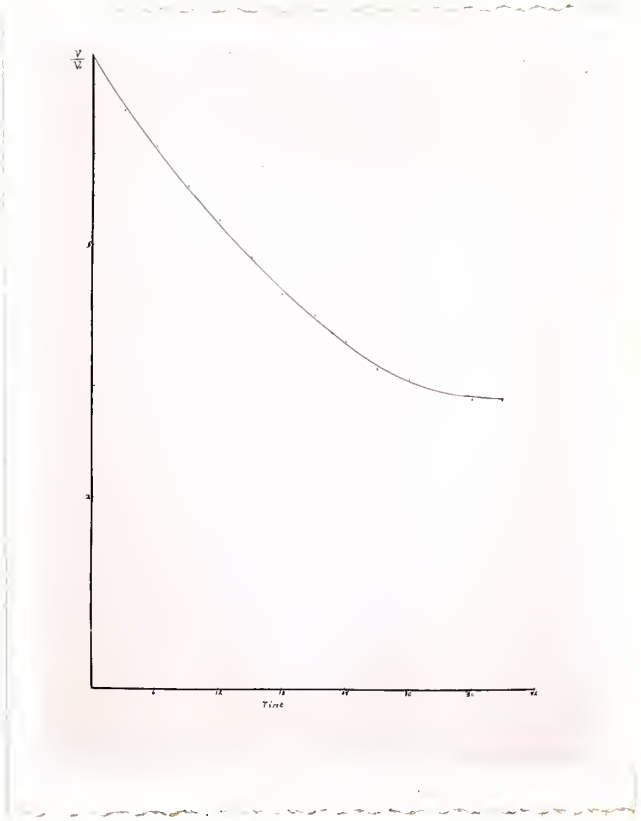

Figure 4: Resorption Curve for 50% Serum.

TABLE 1

Half Resorption Time of Isotonic Saline, 10% and 50% Protein Series

Saline	10% Serum	50% Serum
9.0 sec.	13.5 sec.	14.4 sec.
9.3	12.0	17.7
9.3	11.5	15.0
9.6	11.0	13.9
10.3	10.5	14.2
10.3	11.2	15.0
10.3		14.1
10.5		17.4
10.2		13.8
Mean 9.9±.5 sec.	11.6±1.1 sec.	15.1±1.5 sec.

TABLE 2

Rate of Resorption of 10% Serum at Various Time Intervals

All figures in units of 10^{-4} $mm^3/mm^2/sec.$

Series	1	2	3	4	5	6	Mean
0-15 sec.	5.1	5.1	5.0	5.0	4.6	4.3	4.8 ± .3
15-18	5.1	5.1	4.2	4.6	4.6	3.4	4.7 ± .6
18-21	4.5	5.1	3.4	4.2	4.2	3.0	4.1 ± .7
21-24	3.4	5.1	3.2	3.8	3.9	2.7	3.7 ± .8
24-27	2.7	3.4	3.0	3.0	3.4	2.4	3.0 ± .4
27-30	2.0	2.7	2.7	2.3	3.0	2.0	2.5 ± .4
30-33	1.3	1.6	2.2	1.6	2.2	1.6	1.8 ± .4
33-36	.8	.8	1.3	1.0	1.5	1.3	1.1 ± .3
36-39	.4	.4	1.1	.6	1.0	.8	.7 ± .3

TABLE 3

Rate of Resorption of 50% Serum at Various Time Intervals

All figures in units of 10^{-4} $mm^3/mm^2/sec.$

Time	1	2	3	4	5	6	7	8	9	Mean
0-3 sec.	5.1	5.2	5.8	5.8	5.6	4.6	5.0	5.0	5.0	5.2 ± .4
3-6	4.2	3.9	4.2	4.5	4.2	4.2	3.4	4.2	4.2	4.1 ± .4
6-9	3.8	3.4	3.3	3.6	3.4	3.9	3.0	3.8	3.5	3.5 ± .3
9-12	3.4	3.0	3.0	3.0	3.0	3.0	3.0	3.6	3.2	3.1 ± .2
12-15	3.3	2.4	2.7	2.7	2.7	3.0	3.0	3.3	2.9	2.9 ± .3
15-18	3.0	2.2	2.4	2.7	2.7	2.7	3.0	3.0	2.7	2.7 ± .3
18-21	2.7	2.2	2.2	2.4	2.2	2.4	2.7	2.7	2.4	2.4 ± .2
21-24	2.3	2.0	1.9	2.2	1.9	2.2	2.7	2.0	2.3	2.2 ± .3
24-27	2.0	1.8	1.6	1.9	1.6	1.9	2.4	1.5	1.9	1.8 ± .3
27-30	1.9	1.3	1.3	1.3	1.1	1.6	1.6	1.1	1.5	1.4 ± .3
30-33	1.2	1.3	.8	.8	.8	1.1	1.3	.8	.8	1.0 ± .2
33-36	.8	1.2	.5	.3	.5	.8	.8	.4	.4	.6 ± .3
36-39	.3	.8	.3			.3	.4	.4	.2	.3 ± .2
39-42		.4					.2			
42-45										

DISCUSSION

The droplet method of micropuncture is a relatively new technique used to study the physiology of the kidney. Gertz (17) was the first to describe this type of micropuncture study as a direct method of measuring the influx and efflux of fluid in kidney tubules. Many factors may play a part in our determinations using this method which we are as yet unaware of or at least unable to define accurately; however, these same factors probably influence comparative studies of various fluids to the same extent and tend to cancel out. In the initial phase of the experiment the normal anatomy of the kidney is altered by stripping off the capsule, immobilizing the kidney in a plexiglass boat and proceeding to immerse it in oil. These maneuvers, no doubt, have an effect on blood perfusion and we occasionally see microscopic changes indicative of this with engorgement of the tissue, pale regions or tubules in which the lumens aren't visible. Kidneys manifesting any abnormal changes however, were not used. Only those kidneys with uniformly excellent tubular flow, blood perfusion, and without mottling were studied. Puncture of a tubule with a pipette although only with bevelled tip of 10 microns or less, not only destroys some cells and interrupts the continuity of the epithelium but may distort a segment of the tubule being studied. Leakage of fluid from the lumen of the tubule, at which point the puncture is located, is

possible; however, we believe this to be negligible with the use of a bevelled pipette and with subsequent sealing by oil. The tubule is distended with the injection of fluid and this may alter membrane permeability and cellular function. The diameter of injected tubule in each injection, however, is constant within the limits of our measurements (31 microns ± 1.0). Another factor which could give spurious determination of fluid resorption is the movement of fluid from tubule to pipette or visa-versa during measurements of resorption. This factor was counteracted by essentially sealing off the tubule from the fluid being resorbed with an injection of oil following the injection of the fluid under study. The viscosity of castor oil with Sudan Black is much greater than either the saline or serum solutions. A droplet of the oil mixture moves with resorption of fluid and depending on the cohesive and adhesive forces may influence resorption. The cohesive forces of the mineral oil tend to reduce the surface area of the droplet, forcing a column of oil into globular shape which may distend the tubule at that point. The adhesive forces between the tubule epithelium and the oil droplet also would implement a resistance to movement. Measurements of tubular efflux were made when the forces were in a steady state approximately 2 - 3 seconds after the injection of fluid. The initial inertial forces have been overcome by this time and only the more constant cohesive and adhesive forces are affecting the

system. With the resorption of fluid, hydrostatic pressure would decrease in that segment of the tubule, between the two droplets, below normal tubular fluid pressure and could reduce resorption. Another factor may counteract this however. Tubular flow, proportional to the glomerular filtrate but decreased by the proximal tubular resorption, would transmit a pressure to the complex under study. With the distal droplet resisting flow greater than tubular fluid, tubular flow would increase the hydrostatic pressure of the fluid intervening between the two oil droplets and tend to augment resorption. The distance from the glomerulus to the site of puncture is a negligible factor to be considered for even though as one moves distally along the tubule, tubular flow decreases, tubular pressure remains a constant 12 mm Hg (18).

The factors of altered tubular structure, compromised cellular function from the artificial conditions imposed, and the possibility of pressure changes foreign to normal kidney physiology, may affect an individual determination of fluid resorption. The calculated rate of efflux of fluid may be different from the actual value in the intact animal; however, this may not invalidate conclusions drawn from comparative studies of various fluids studied under the same artificial conditions.

A formula can be derived expressing the rate of resorption as a function of the observed volume change between the two

oil droplets, if we assume a constant tubular diameter. Comparing the diameter as observed in the pictures of injected tubules we have found the standard deviation to be approximately ± 1 micron with a mean diameter of 31 microns.

I) $V = \pi r^2 l$

$l = V/\pi r^2$ $\qquad$ l = distance between oil droplets
r = radius of tubule

$F = 2\pi rl$

where F = area of tubule between oil droplets

$$F = 2\pi r \frac{V}{\pi r^2} = \frac{2V}{r}$$

II) ϕ is defined as the amount of fluid absorbed in mm^3 per unit area of tubular epithelium per second.

$$\therefore -\frac{dV}{dt} = \phi F$$

$$-\frac{dV}{dt} = \phi \frac{2V}{r}$$

$$\phi\, dt = \frac{dV}{V} \qquad V/2$$

III) $$-\frac{\phi t2}{r} = (\ln V) \qquad +C$$

$$\phi = \ln V + C$$

$$\therefore -\frac{\phi t2}{r} = \ln V - \ln V_2$$

$$\phi = -\frac{r}{2} \frac{\ln V/V_o}{t}$$

This equation is used to calculate ϕ over a 3 second interval where V_1 is the beginning volume and V_2 the terminating volume.

Although $\emptyset$ changes from one instant to the next for protein solutions we may use this knowing that over the 3 second interval $\emptyset$ as calculated is the average resorption.

IV) when $T = t_{\frac{1}{2}}$ (half time resorption) $\frac{V}{V_o} = .5$

$$\therefore \quad \emptyset = \frac{-V \ln .5}{2\, t_{\frac{1}{2}}} = \frac{-r\,(-.6932)}{2\, T_{\frac{1}{2}}} = \frac{r}{t_{\frac{1}{2}}} .347$$

$\emptyset$ remains constant with saline solution. Therefore, we can calculate $\emptyset$ knowing the half time resorption. Gertz (17) has determined the diameter of the tubule to be 31 ± .5 microns. Of a series of twenty tubular measurements in the present work the diameter was 31 ± 1.0 microns.

In a series of nine, we found the half time resorption of a saline solution containing 150 mEq/L to be 9.9 ± .5 seconds which is in close agreement with the value obtained by Gertz (17). The half time resorption of 11.6 ± 1.1 seconds for 10% serum and 15.1 ± 1.5 seconds for 50% serum indicates that the proteins had some affect on the resorption of the fluid and that with increased protein concentration the half time is prolonged. However, if we focus on the initial period of resorption of a protein solution the rate of resorption of a 50% serum, $\emptyset = (5.2 \pm .4)\ 10^{-4}$ mm^3/mm^2 seconds is quite similar to the constant rate of $\emptyset = (5.4 \pm .3)$ $10^{-4}/m^3/m^2/$seconds for isotonic saline. When we take into account the lapse of time of 2 - 3 seconds between injection of fluid and photographing the distance between droplets for the determination

of volume V_o the solution had a protein concentration greater than that of 50% serum.

Tubular fluid has normally very little protein content when compared with plasma (8). Thus, with the hydrostatic pressures of peritubular capillaries and proximal tubular lumens being equal as shown by Gottschalk and Mylle (18), it becomes apparent that the protein in the plasma may exert an effective osmotic force favoring the resorption of water and salt from the proximal tubules. The question is thus posed as to how much oncotic pressure accounts for tubular fluid reabsorption in the proximal tubule. The normal plasma oncotic pressure is 25 mm of Hg and with a filtration fraction of approximately 20%, the oncotic pressure in the peritubular capillaries increases to 30 - 32 mm of Hg. It is suggested by Vander et al (19) that an even greater increase in peritubular capillary oncotic pressure occurs in congestive heart failure and this accounts for the decreased salt and water loss in such patients, the effects of which lead to edema. Heller and Jacobson (20) have studied people in the edematous state and found the filtration fraction to be increased from the normal of .174 ± .023 to .405 ± .083 explicable by the decrease in renal blood flow. In these states the oncotic pressure in the peritubular capillaries would be approximately 40 mm of Hg. If oncotic pressure is a major mechanism in explaining proximal tubular salt and water reabsorption,

then the observed increase in filtration fraction would be a formidable factor in elucidating the physiology of decreased salt and water loss in edematous states.

In view of the present work, the theory that oncotic pressure is a significant force in proximal tubular reabsorption of salt and water is untenable. By introducing a fluid into the proximal tubule containing a protein concentration 50% that of serum, the effective oncotic pressure is essentially reduced 50% or to approximately 13 mm Hg and probably a little more when the first determination is made. At this protein concentration the initial decrement of tubular fluid is approximately the same as when isotonic saline was studied. The comparable rates of resorption of saline, $\emptyset$ being $(5.4 \pm .3)$ 10 $mm^3/mm^2/sec$ and $\emptyset$ being $(5.2 \pm .4)\ 10^{-4}$ $mm^3/mm^2/sec$ for 50% serum during the first 3 seconds of resorption means that at least a 50% reduction of plasma proteins has little if any effect on proximal tubular resorption. And this would indicate that oncotic pressure as a mechanism contributing to proximal tubular resorption is of minimal importance.

During the 3 - 6 second interval of study (Table 2), the rate of resorption for the protein solution (originally 50% serum) decreased to $(4.1 \pm .4)\ 10^{-4}$ $mm^3/mm^2/sec$. Subsequently the rate decreased (Table 2) until the rate was $(.3 \pm .2)\ 10^{-4}$ $mm^3/mm^2/sec$

during the 36 - 39 second interval. This decrease in rate of resorption as the volume decreases, could be due either to an increase in protein concentration associated with the more rapid reabsorption of salt and water finally reaching a concentration gradient against which further resorption is impossible, or to "poisoning" of the cells by the high concentration of protein. The high concentration of protein in the tubular fluid may "overload" the cells and affect the transport system for salt. We have no evidence to invoke either of these theories to explain the fall-off of resorption of a protein solution. To elucidate this problem, it would be helpful to measure the concentration of the protein in the residual tubular fluid after injection and resorption, to determine protein concentration in the tubular cells of the segment under study possibly by histochemical methods, and to follow a protein injection with a saline injection into the same segment to analyze whether the reabsorbing power of the cells have been altered.

SUMMARY

Proximal tubular resorption was studied in the rat kidney using the droplet method of micropuncture as described by Gertz. Solutions of isotonic saline, 10% rat serum and 50% rat serum were injected into the tubules and resorption observed and recorded by photographic methods. The initial rates of resorption of the protein solutions were shown to be equal to the constant resorption rate of isotonic saline. The rate of resorption of the protein solution decreased with progression of time until equilibrium was reached and a small amount of fluid remained in the tubule. The results of comparable equal rates of saline resorption and initial rates of protein resorption indicate that a 50% decrease in plasma protein would have essentially no effect on proximal tubular resorption and that peritubular capillary oncotic pressure is not a major factor in explaining the mechanism of salt and water resorption in the proximal tubule. The resorption rate decrease with time and tubular fluid volume decrease is probably due either to overloading of the tubular cells with protein which inhibits active resorption or a high protein concentration gradient between tubule and plasma or both.

BIBLIOGRAPHY

1. Cushny, A.R.: Journal of the Secretion of Urine, 1926, London.
2. DeHaan, J.: The renal function as judged by excretion of vital dye-stuff, J. Physiology, 56:444, 1922.
3. Morner, K.A.H.: Untersuchungen über die Proteinstoffe and die Eiweissfullenden Substanzen des Normale Menschenharne, Scand. Arch. J. Physiologie, 6:332, 1895.
4. Addis, T.: Proteinuria and cylindruria, Proc. Calif. Acad. Med., 2:38, 1931-32.
5. Dock, W.: Proteinuria and associated renal changes, N.E.J.M., 227:633, 1942.
6. Bayliss, L.E., Kerridge, P.M., and Russell, D.S.: The excretion of protein by the mammalian kidney, J. Physiology, 77: 386, 1935.
7. Walker, A.M., Bott, P.A., Oliver, J., and MacDowell, M.C.: Am. J. Physiol., 134:580, 1941.
8. Sellers, A.L.: The mechanisms and significance of protein excretion by the normal kidney, Arch. Int. Med., 98:801, 1956.
9. Ekehorn, G.: On principle of renal functions, Acta. Med. Scand. Supp., 36, 1931.
10. Monke, J.V., and Yuile, C.L.: The renal clearance of hemoglobin in dog, J. Exp. Med., 72:149, 1940.
11. Lichty, J.A., Havell, W.H., and Whipple, G.W.: Renal threshold for hemoglobin in dogs, J. Exp. Med., 55:603, 1932.

12. Gibson, S.B.: Proc. Soc. Exper. Biol. and Med., 72:608, 1949.

13. Sellers, A.L., Griggs, N., Marmoisten, J. and Goodman, H.C.: J. Exp. Med., 100:1, 1954.

14. Oliver, J., MacDowell, M., and Lee, Y.C.: Cellular mechanism of protein metabolism in nephron, J. Exp. Med., 99:589, 1954.

15. Oliver, J., Moses, M.J., MacDowell, M., and Lee, Y.C.: Cellular mechanism of protein metabolism in the nephron, J. Exp. Med., 99:605, 1954.

16. Schitler, A.A. and Schayer, R.W., and Hess, E.L., J. Gen. Physiol., 36:489, 1953.

17. Gertz, K.H.: Transtubuläre Natriumchlorid flusse und Pernenbilität für Nichtelektrolyte im proximalen und. distalen Kowvolut der RaHenniere, Pflügers Archiv., 276:336-356 (1963).

18. Gottschalk, C.W., and Mylle, M.: Micropuncture study of pressures in proximal tubule and peritubular capillaries of rat kidney and their relation to arterial and renal venous pressures, Am. J. Physiol., 185:430, 1956.

19. Vander, A.J., Malvin, R., Wilde, W.S., Sullivan, L.P.: Editorial, Re-examination of salt and water retention in congestive heart failure, Am. J. Med., 24:497-502, 1958.

20. Heller, B.I., and Jacobson, W.E.: Renal hemodynamics in heart disease, Am. Heart J., 39:188, 1950.

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